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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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**Blatt 2 der Bescheinigung
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Helicobacter pylori live vaccine

Specification

5 The present invention relates to novel recombinant live vaccines, which provide protective immunity against an infection by Helicobacter pylori and a method of screening H. pylori antigens for optimized vaccines.

10 Helicobacter is a gram-negative bacterial pathogen associated with the development of gastritis, peptic ulceration and gastric carcinoma. Several Helicobacter species colonize the stomach, most notably H. pylori, H. heilmanii and H. felis.
15 Although H. pylori is the species most commonly associated with human infection, H. heilmanii and H. felis also have been found to infect humans. High H. pylori infection rates are observed in third world countries, as well as in industrialized countries. Among all the virulence factors described in H.
20 pylori, urease is known to be essential for colonisation of gnotobiotic pigs and nude mice. Urease is an enzyme composed of two structural subunits (UreA and UreB). Previous studies have indicated that oral immunization using recombinant UreB plus cholera toxin were able to protect mice from gastric colonisa-
25 tion with H. felis and H. pylori (Michetti et al., Gastroenterology 107 (1994), 1002-1011). By oral administration of recombinant UreB antigens, however, in several cases only an incomplete protection can be obtained. Other H. pylori antigens shown to give partial protection are the 87 kD vacuolar
30 cytotoxin VacA (Cover and Blaser, J. Biol. Chem. 267 (1992), 10570; Marchetti et al., Science 267 (1995), 1655) and the 13 and 58 kD heat shock proteins HspA and HspB (Ferrero et al., Proc. Natl. Acad. Sci. USA 92 (1995), 6499).

35 Attenuated pathogens, e.g. bacteria, such as Salmonella, are known to be efficient live vaccines. The first indications of the efficacy of attenuated Salmonella as good vaccine in hu-

mans came from studies using a chemically mutagenized *Salmonella typhi* Ty21a strain (Germanier and Furer, J. Infect. Dis. 141 (1975), 553-558), tested successfully in adult volunteers (Gilman et al., J. Infect. Dis. 136 (1977), 717-723) and later 5 on in children in a large field trial in Egypt (Whadan et al., J. Infect. Dis. 145 (1982), 292-295). The orally administered Ty21a vaccine was able to protect 96% of the Egyptian children vaccinated during three years of surveillance. Since that time new attenuated *Salomonella* live vector vaccines have developed 10 (Hone et al., Vaccine 9 (1991), 810-816), in which well defined mutations incorporated into the chromosome gave rise to non-virulent strains able to induce strong immune responses after oral administration (Tacket et al., Vaccine 10 (1992), 443-446 and Tacket et al., Infect. Immun. 60 (1992), 536-541). 15 Other advantages of the live attenuated *Salmonella* vaccine include its safety, easy administration, long-time protection and no adverse reactions in comparison with the former inactivated wholesale typhoid vaccines (Levine et al., Typhoid Fever Vaccines. In: Plotkin S.A., Mortimer E.A. Jr. (eds.) Vaccines. 20 Philadelphia: WB Saunders (1988), 333-361).

Mutants of *S. typhimurium* have been extensively used to deliver antigens because of the possibility to use mice as an animal model, which is believed to mimick *S. typhi* infections 25 in humans. The attenuation of *S. typhimurium* most commonly used consists in site directed mutagenesis of genes affecting the synthesis of aromatic amino acids. Such strains, designated aro mutants, have a negligible pathogenicity, as demonstrated in animal models and human trials using these constructs 30 (Hoiseth and Stocker, Nature 291 (1981), 238-239; Tacket et al. (1992), Supra). Advantage has been taken from the potent immunogenicity of live *Salmonella* vaccine to deliver heterologous antigens. Expression of specific antigens in attenuated *Salmonella* has conferred murine protection against several 35 bacterial pathogens. The use of recombinant live vaccines, which are capable of expressing *Helicobacter* antigens and protecting the vaccinated animals, has not yet been described.

The use of attenuated live vaccines for the treatment of a Helicobacter infection has also not been rendered obvious. The reason therefor being that in the course of the Helicobacter infection a strong immune response against the pathogen per se is induced, which, however, does not lead to a protective immunity. Thus, it was highly surprising that a protective immune response is achieved when using recombinant attenuated bacterial cells as antigen carriers, which are capable of expressing a DNA molecule encoding a Helicobacter antigen.

10 Apparently, recombinant attenuated bacterial cells expressing a Helicobacter antigen are capable of creating a qualitatively different immune response against the heterologous Helicobacter antigen than Helicobacter itself does against its own homologous antigen. Surprisingly, a non-protective immune

15 response is thus transformed into an immune response protecting against Helicobacter infections. This unexpected observation renders it possible to use recombinant attenuated pathogens, e.g. bacterial cells, particularly Salmonella, as carriers for the screening of protective antigens, to apply the

20 protective antigens identified in this manner in any vaccine against Helicobacter infections, and to use recombinant attenuated bacteria as carriers of protective antigens for the immunization against Helicobacter infections in humans and other mammals.

25

Thus, a subject matter of the present invention is a recombinant attenuated pathogen, which comprises at least one heterologous nucleic acid molecule encoding a Helicobacter antigen, wherein said pathogen is capable to express said nucleic acid

30 molecule or capable to cause the expression of said nucleic acid in a target cell. Preferably the nucleic acid molecule is a DNA molecule.

The attenuated pathogen is a microorganism strain which is

35 able to cause infection and preferably effective immunological protection against the actual pathogen but is no longer pathogenic per se. The attenuated pathogen can be a bacterium, a

virus, a fungus or a parasite. Preferably it is a bacterium; e.g. *Salmonella*, such as *S. typhimurium* or *S. typhi*, *Vibrio cholerae* (Mekalanos et al., *Nature* 306 (1983), 551-557), *Shigella* Species such as *S. flexneri* (Sizemore et al., *Science* 270 (1995), 299-302; Mounier et al., *EMBO J.* 11 (1992), 1991-1999), *Listeria* such as *L. monocytogenes* (Milon and Cossart, *Trends in Microbiology* 3 (1995), 451-453), *Escherichia coli*, *Streptococcus*, such as *S. gordonii* (Medaglini et al., *Proc. Natl. Acad. Sci. USA* 92 (1995) 6868-6872) or *Mycobacterium*, such as *Bacille Calmette Guerin* (Flynn, *Cell. Mol. Biol.* 40 Suppl. 1 (1994), 31-36). More preferably the pathogen is an attenuated enterobacterium such as *Vibrio cholerae*, *Shigella flexneri*, *Escherichia coli* or *Salmonella*. Most preferably the attenuated pathogen is a *Salmonella* cell, e.g. a *Salmonella* aro mutant cell. The attenuated pathogen, however, can be a virus, e.g. an attenuated *vaccinia* virus, *adenovirus* or *poxvirus*.

The nucleic acid molecule which is inserted into the pathogen codes for a *Helicobacter* antigen, preferably a *H. felis*, *H. heilmanni* or *H. pylori* antigen, more preferably a *H. pylori* antigen. The *Helicobacter* antigen can be a native *Helicobacter* polypeptide, an immunologically reactive fragment thereof, or an immunologically reactive variant of a native polypeptide or of a fragment thereof. Further, the *Helicobacter* antigen can be a protective carbohydrate or a peptide mimotope simulating the three-dimensional structure of a native *Helicobacter* antigen. Peptide mimotopes can be obtained from peptide libraries presented on the surface of bacterial cells (cf. PCT/EP96/01130). Of course, the transformed cell can also contain several DNA molecules coding for different *Helicobacter* antigens.

Attenuated bacteria can be used to transcribe and translate said nucleic acid molecule directly in the bacterial cell or to deliver said nucleic acid molecule to the infected target cell, such that the DNA molecule is transcribed and/or trans-

lated by the eukaryotic target cell machinery. This indirect bacterial vaccination procedure, termed here as genetic vaccination, has been successfully used with Shigella as a carrier (Sizemore, D. R., Branstrom, A. A. & Sadoff, J. C. (1995) 5 Attenuated Shigella as a DNA delivery vehicle for DNA-mediated immunization. *Science* 270:299-302).

In a preferred embodiment of the present invention the Helicobacter antigen is urease, a urease subunit or an immunologically reactive variant or fragment thereof or a peptide mimotope thereof. In a further preferred embodiment of the present invention the Helicobacter antigen is a secretory polypeptide from Helicobacter, an immunologically reactive variant or fragment thereof or a peptide mimotope thereof. A 10 process for identifying Helicobacter genes coding for such secretory polypeptides, and particularly for adhesins, has been disclosed in the international patent application PCT/EP96/02544, which is incorporated herein by reference. A 15 This process comprises

- 20 a) preparing a gene bank of *H. pylori* DNA in a host organism containing an inducible transposon coupled to a marker of secretory activity,
- b) inducing the insertion of the transposon into the *H. pylori* DNA and
- 25 c) conducting a selection for clones containing a secretory gene by means of the marker, and optionally further
- d) conducting a retransformation of *H. pylori* by means of the DNA of clones containing genes having secretory activity, wherein isogenic *H. pylori* mutant strains are produced by means of integrating the DNA into the chromosome, and
- 30 e) conducting a selection detecting adherence-deficient *H. pylori* mutant strains.

35 Suitable examples of antigens obtainable by the above process are selected from the group consisting of the antigens AlpA, AlpB, immunologically reactive variants or fragments thereof

or peptide mimotopes thereof. The nucleic and amino acid sequences of the antigens AlpA and AlpB have been disclosed in the international patent applications PCT/EP96/02545 and PCT/-EP96/04124, which are incorporated herein by reference. Further, the nucleic and amino acid sequences of AlpB are shown in SEQ ID NO. 1 and 2, and the nucleic and amino acid sequences of AlpA in SEQ ID NO. 3 and 4.

It is also conceivable, however, that an intracellular antigen 10 is used which can be presented on the surface, e.g. by autolytic release, and confers immunological protection.

The presentation of the Helicobacter antigens in the recombinant pathogen according to the invention can be accomplished 15 in different ways. The antigen or the antigens can be synthesized in a constitutive, inducible or phase variable manner in the recombinant pathogen. Concerning the constitutive or inducible synthesis of the Helicobacter antigens known expression systems can be referred to, as have been described by 20 Sambrook et al., Molecular Cloning, A Laboratory Manual (1989), Cold Spring Harbor Laboratory Press.

Particularly preferred the antigens are presented in a phase variable expression system. Such a phase variable expression 25 system for the production and presentation of foreign antigens in hybrid live vaccines is disclosed in EP-B-0 565 548, which is herein incorporated by reference. In such a phase variable expression system the nucleic acid molecule encoding the Helicobacter antigen is under control of an expression signal, 30 which is substantially inactive in the pathogen, and which is capable of being activated by a spontaneous reorganization caused by a nucleic acid, e.g. DNA reorganization mechanism in the pathogen, e.g. a specific DNA inversion process, a specific DNA deletion process, a specific DNA replication process 35 or a specific slipped-strand-mispairing mechanism.

A recombinant cell having a phase variable expression system

is capable of forming two subpopulations A and B, wherein the division into said subpopulations occurs by spontaneous reorganization in the recombinant nucleic acid, wherein said subpopulation A is capable of infection and immunologically active per se, while subpopulation B, which is regenerated from subpopulation A, produces at least one heterologous Helicobacter antigen and acts immunologically with respect to said additional antigen.

10 The activation of the expression signal encoding the Helicobacter antigen can be directly accomplished by nucleic acid reorganization or, alternatively, indirectly accomplished by activation of a gene encoding a protein which controls the expression of the gene encoding the Helicobacter antigen. The
15 indirect activation represents a system which allows the production of the Helicobacter antigen via a cascade system, which can be realized e.g. in that the gene directly controlled by DNA reorganization codes for an RNA polymerase which is specific for the promoter preceding the Helicobacter gene, or
20 a gene regulator which in another specific manner induces the expression of the Helicobacter gene. In an especially preferred embodiment of the present invention the expression signal for the gene encoding the Helicobacter antigen is a bacteriophage promoter, e.g. a T3, T7 or SP6 promoter, and the activation
25 of the expression signal is caused by a nucleic acid reorganization resulting in the production of a corresponding bacteriophage RNA polymerase in the pathogen.

The phase variable expression system can be adjusted to provide a preselected expression level of the Helicobacter antigen. This can be accomplished e.g. by modifying the nucleotide sequence of the expression signal, which is activated by the nucleic acid reorganization mechanism, and/or by inserting further genetic regulation elements.

35

The Helicobacter antigens can be produced in an intracellular, as well as in an extracellular manner in the pathogen accord-

ing to the invention. For instance, autotransporter systems such as the IgA-protease system (cf. for instance EP-A-0 254 090) or the *E. coli* AIDA-1 adhesin system (Benz et al., Mol. Microbiol. 6 (1992), 1539) are suited as extracellular secretory system. Other suitable outer membrane transporter systems are the RTX-toxin transporters, e.g. the *E. coli* hemolysin transport system (Hess et al., Proc. Natl. Acad. Sci. USA 93 (1996), 11458-11463).

10 The pathogen according to the invention can contain a second heterologous nucleic acid, e.g. DNA molecule, which codes for an immunomodulatory polypeptide influencing the immune response quantitatively or qualitatively, apart from the nucleic acid molecule encoding the *Helicobacter* antigen. Examples of 15 such immunomodulatory polypeptides are immune-stimulating peptides, cytokines like IL-2, IL-6 or IL-12, chemokines, toxins, such as cholera toxin B or adhesins.

The present invention also refers to a pharmaceutical composition comprising as an active agent a recombinant attenuated pathogen as described above, optionally together with pharmaceutically acceptable diluents, carriers and adjuvants. Preferably, the composition is a living vaccine. The vaccination routes depend upon the choice of the vaccination vector. The 25 administration may be achieved in a single dose or repeated at intervals. The appropriate dosage depends on various parameters such as the vaccinal vector itself, or the route of administration. Administration to a mucosal surface (e.g. ocular, intranasal, oral, gastric, intestinal, rectal, vaginal or 30 urinary tract) or via the parenteral route (e.g. subcutaneous, intradermal, intramuscular, intravenous or intraperitoneal) might be chosen. A method for the preparation of the living vaccine comprises formulating the attenuated pathogen in a pharmaceutically effective amount with pharmaceutically acceptable diluents, carriers and/or adjuvants.

Further, the present invention refers to a method for prepar-

ing a recombinant attenuated pathogen as defined above, comprising the steps of a) inserting a nucleic acid molecule encoding a Helicobacter antigen into an attenuated pathogen, wherein the recombinant pathogen, e.g. a transformed bacterial cell, is obtained, which is capable of expressing said nucleic acid molecule or is capable to cause expression of said nucleic acid molecule in a target cell and b) cultivating said recombinant attenuated pathogen under suitable conditions. If the pathogen is a bacterial cell, the nucleic acid molecule encoding the Helicobacter antigen can be located on an extra-chromosomal plasmid. It is, however, also possible to insert the nucleic acid molecule into the chromosome of the pathogen.

Furthermore, the present invention refers to a method for identifying Helicobacter antigens which raise a protective immune response in a mammalian host, comprising the steps of: a) providing an expression gene bank of Helicobacter in an attenuated pathogen and b) screening the clones of the gene bank for the ability to confer a protective immunity against a Helicobacter infection in a mammalian host. Preferably, this identification process takes place in a phase variable expression system, rendering possible a stable expression of all of the Helicobacter antigens. Recombinant clones can then be applied as "pools" for the oral immunization of test animals, such as mice. The potential of these clones as protective antigen is then determined via a challenge infection with Helicobacter, e.g. a mouse-adapted H. pylori strain. Thus, there is a possibility of directly selecting optimized H. pylori vaccine antigens.

30

The invention will be further illustrated by the following figures and sequence listings.

Fig. 1: shows a schematic illustration of the urease expression vector pYZ97; whereon the genes coding for the urease subunits UreA and UreB are located under transcriptional control of the T7 promoter φ10.

There is a ribosomal binding site (RBS) between the T7 promoter and the urease genes. Further, the plasmid exhibits an origin of replication (ori), a β -lactamase resistance gene (bla) and 4 T7 terminators in series.

5

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Apart from the expression by the T7 promoter, a constitutive low level expression of the urease A and B subunits can also be brought about via a cryptic promoter, which is located upstream from the T7 promoter, on the plasmid pYZ97.

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Fig. 2: shows a schematic illustration of the T7 RNA polymerase (T7RNAP) expression cassettes pYZ88, pYZ84 and pYZ114, which can be integrated into the chromosomes of bacteria.

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In the high-expression cassette pYZ88 the lambda PL promoter is located in inverse orientation, upstream from the T7RNAP gene. A gene for the temperature-sensitive repressor cI 857 (cI) is under control of this promoter. A terminator of the bacteriophage fd (fdT) is situated upstream from the cI gene. The gin gene (Mertens, EMBO J. 3 (1984), 2415-2421) codes for a control enzyme of a DNA reorganization mechanism. A DNA sequence coding for the tRNA Arg is located downstream from the gin gene.

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In phase A the PL promoter responsible for the expression of the T7RNAP gene is directed in the direction of the cI857 gene and the gin gene. The consequence of this is that an active repressor is formed at the permissive temperature of 28°C and reduces the transcription from the PL promoter. At a higher temperature the transcription of the PL promoter is increased, since the repressor is inactivated at least partially under such external influences. The

temperature-dependent increase in the transcription also causes a corresponding increase in the expression of the following gin gene, which as a control enzyme catalyses the inversion of the PL promoter and the transition in phase B, in which the T7RNAP gene is expressed.

In the high-expression system pYZ88 a further fdT transcription terminator is located between a kanamycin-resistance gene (km) and the promoter of this gene. In this manner, the synthesis of an anti-sense RNA, inversely orientated to the T7RNAP gene, which normally contributes to the reduction of the T7RNAP expression, is reduced. This results in a high expression of the T7RNAP.

In the medium-expression system pYZ84 a transcription terminator (fdT) is located between the PL promoter and the start of the T7RNAP gene. In this manner the expression of the T7RNAP mRNA is reduced. Additionally, the anti-sense RNA affects the T7RNAP translation. Therefore, only a medium expression occurs.

In the low-expression system pYZ114 a deletion of 100 bp in PL is additionally introduced (Δ PL). In this manner the activity of the PL promoter is reduced to a high extent, which leads to a lower T7RNAP expression and thus to a reduction of the UreA/B gene expression. In this construct the effect of the cryptic promoter on pYZ97 is already observed.

SEQ ID NO. 1 and 2 show the nucleotide sequence of the adhesin gene AlpB from *H. pylori* and the amino acid sequence of the polypeptide coded therefrom.

SEQ ID NO. 3 and 4 show the nucleotide sequence of the adhesin

gene AlpA from *H. pylori* and the amino acid sequence of the protein coded therefrom.

Experimental part

5

Materials and Methods

Bacterial strains: *S. typhimurium* SL3261 live vector vaccine strain was used as a recipient for the recombinant *H. pylori* urease plasmid constructs. *S. typhimurium* SL3261 is an aroA transposon mutant derived from *S. typhimurium* SL1344 wild type strain. *S. typhimurium* SL3261 is a non-virulent strain that gives protection to mice against infection with wild type *S. typhimurium* after oral administration (Hoiseth and Stocker 1981) Supra). *S. typhimurium* SL3261 and derivatives thereof, which contain the urease expression plasmid pYZ97 (extrachromosomal) and the T7RNAP expression cassettes pYZ88, pYZ84 or pYZ114, respectively (integrated into the chromosome) are indicated in table 1. Luria broth or agar was used for bacterial growth at 28°C. *H. pylori* wild type strain grown at 37°C on serum plates was used for the challenge experiments.

Immunization of mice: Four weeks Balb/c mice purchased from Interfauna (Tuttlingen, Germany) were adapted two weeks in an animal facility before being used for experimentation. 150 µl of blood was taken retroorbitally from all mice to obtain preimmune serum. Retroorbital bleedings were repeated from all immunized mice 1 week and 3 weeks after immunization.

30 Eight groups of 5 mice including controls were used in this study (table 2). Group A, the naive control group, was not immunized with *Salmonella* neither challenged with wild type *H. pylori*. The rest of the groups were all orally immunized. Group B, a negative control group, did not receive *Salmonella* and was challenged with *H. pylori*. Mice from groups C to G were immunized with *Salmonella* vaccine strains and challenged with *H. pylori*. The last group H received recombinant urease B

in combination with cholera toxin and was also challenged.

Prior to immunizations mice were left overnight without solid food and 4 hours without water. 100 μ l of 3% sodium bicarbonate were given orally using a stainless steel catheter tube to neutralize the stomach pH. Then mice from group B received 100 μ l PBS and mice from groups C to G received 1.0×10^{10} CFU of *Salmonella* in a 100 μ l volume. Mice from group H received four times 100 μ l of a mixture of recombinant *H. pylori* UreaseB plus cholera toxin, one dose every week. After every immunization water and food were returned to the mice.

H. pylori challenge: Four weeks after the first oral immunization mice from groups B to H were challenged with *H. pylori*.
15 Mice were left overnight without solid food and without water 4 hours prior to the challenge. 100 μ l of 3% sodium bicarbonate were given orally to the mice using a stainless steel catheter tube, followed by an oral dose of 5.0×10^9 CFU/ml of *Helicobacter pylori*. Water and food were returned to the mice
20 after the challenge.

Collection of blood and tissues from mice: Twelve weeks after the first immunization the mice were left overnight without food and subsequently sacrificed for analysis of protection
25 and immune response. The mice were anaesthetized with Metoxyfluorane for terminal cardiac bleeding and prior to sacrifice by cervical dislocation. Under aseptic conditions, spleen and stomach were carefully removed from each mouse and placed on ice in separate sterile containers until further processing.
30 Large and small intestine were obtained for further isolation of the intestinal fluid.

Processing of stomach and measurement of urease activity: The degree of *H. pylori* colonisation in the mouse stomach was
35 measured by the presence of active urease in the tissue. The Jatrox-test (Röhm-Pharma GmbH, Weiterstadt, Germany) was used according to the suppliers' directions. Stomach mucosa was

exposed and washed with PBS, half of the antral portion of the stomach was immediately placed inside an Eppendorf tube containing the substrate for measurement of urease activity. Absorbance at 550 nm was measured after tubes were incubated for 4 hours at room temperature. The rest of the stomach tissue was stored at -20°C for further treatments. The urease activity values obtained from the stomach of naive mice, which did not undergo immunization or challenge, were used to create a base line to indicate the absence of *H. pylori* infection and therefore protection.

Table 1
15 UreA and UreB expressing *S. typhimurium* vaccine strains

Strains	Urease Expression	Source
<i>S. typhimurium</i> SL3261	Negative	Hoiseth and Stocker
<i>S. typhimurium</i> SL3262 pYZ97	Constitutive Low	this study
<i>S. typhimurium</i> SL3261::pYZ88pYZ97	High T7-induced expression	this study
<i>S. typhimurium</i> SL3261::pYZ84pYZ97	Medium T7-induced expression	this study
<i>S. typhimurium</i> SL3261::pYZ114pYZ97	Low T7-induced expression	this study

Table 2

Mice groups used for immunization

Group	Immunogen	No. of oral immunizations
A	None	0
5 B	PBS oral immunization	1
C	S. typhimurium S3261	1
D	S. typhimurium S3261 pYZ97	1
E	S. typhimurium S3261::pYZ88pYZ97	1
F	S. typhimurium S3261::pYZ84pYZ97	1
10 G	S. typhimurium S3261::pYZ114pYZ97	1
H	Urease B plus cholera toxin	4

15 Results:

In the control mice (groups B and C) 100% infection with *H. pylori* was observed. In the mice immunized with recombinant attenuated pathogens (groups D, E, F, G) between 0% and 60% 20 infection (100% to 40% protection) was observed. Immuno-protection did not correlate with humoral anti-UreA and UreB response, suggesting that, in addition to humoral immunity, cellular immunity is critical for protection against *H. pylori* infection. The results indicate that oral immunization of mice 25 with UreA and UreB delivered by *S. typhimurium* attenuated strain is effective to induce high levels of protection against *H. pylori* colonisation.

In the mice immunized with recombinant urease B plus cholera 30 toxin considerably higher levels of urease activity were ob-

served under said experimental conditions than when administering the recombinant attenuated pathogens according to the invention.

s The results of the urease test have been illustrated in table
3.

Table 3

Group	Mouse	$E_{550\text{nm}, 4h}$	$E_{4h} - E_{\text{control}}$	$E_{\text{corr.}} \cdot 3$	Dilution
A	1	0,085	-0,022	-0,066	200 μl +400 μl
	2	0,091	-0,016	-0,048	200 μl +400 μl
	3	0,116	0,009	0,027	200 μl +400 μl
	4	0,099	-0,008	-0,024	200 μl +400 μl
	5	0,101	-0,006	-0,018	200 μl +400 μl
Control		0,107	0	0	200 μl +400 μl
B	1	0,394	0,292	0,876	200 μl +400 μl
	2	0,464	0,362	1,086	200 μl +400 μl
	3	0,329	0,227	0,681	200 μl +400 μl
	4	0,527	0,425	1,275	200 μl +400 μl
	5	0,462	0,36	1,08	200 μl +400 μl
Control		0,102	0	0	200 μl +400 μl
C	1	0,248	0,145	0,435	200 μl +400 μl
	2	0,369	0,266	0,798	200 μl +400 μl
	3	0,209	0,106	0,318	200 μl +400 μl
	4	0,219	0,116	0,348	200 μl +400 μl
	5	0,24	0,137	0,411	200 μl +400 μl
Control		0,103	0	0	200 μl +400 μl
D	1	0,143	0,002	0,004	300 μl +300 μl
	2	0,156	0,015	0,03	300 μl +300 μl
	3	0,142	0,001	0,002	300 μl +300 μl
	4	0,114	-0,027	-0,054	300 μl +300 μl
	5	0,133	-0,008	-0,016	300 μl +300 μl
Control		0,141	0	0	300 μl +300 μl
E	1	0,127	0,027	0,081	200 μl +400 μl
	2	0,094	-0,006	-0,018	200 μl +400 μl
	3	0,099	-0,001	-0,003	200 μl +400 μl
	4	0,161	0,061	0,183	200 μl +400 μl
	5	0,198	0,098	0,294	200 μl +400 μl
Control		0,1	0	0	200 μl +400 μl
F	1	0,166	0,025	0,05	300 μl +300 μl
	2	0,145	0,004	0,008	300 μl +300 μl
	3	0,166	0,025	0,05	300 μl +300 μl
	4	0,154	0,013	0,026	300 μl +300 μl
	5	0,301	0,16	0,32	300 μl +300 μl
Control		0,141	0	0	300 μl +300 μl
G	1	0,084	-0,019	-0,057	200 μl +400 μl
	2	0,087	-0,016	-0,048	200 μl +400 μl
	3	0,269	0,166	0,498	200 μl +400 μl
	4	0,085	-0,018	-0,054	200 μl +400 μl
	5	0,092	-0,011	-0,033	200 μl +400 μl
Control		0,103	0	0	200 μl +400 μl
H	1	0,638	0,531	1,593	200 μl +400 μl
	2	0,282	0,175	0,525	200 μl +400 μl
	3	0,141	0,034	0,102	200 μl +400 μl
	4	0,135	0,028	0,084	200 μl +400 μl
	5	0,171	0,064	0,192	200 μl +400 μl
Control		0,107	0	0	200 μl +400 μl

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Max-Planck-Gesellschaft zur Foerderung der
Wissenschaften e.V. Berlin
(B) STREET: Hofgartenstr. 2
(C) CITY: Muenchen
(E) COUNTRY: Germany
(F) POSTAL CODE (ZIP): 80539

(ii) TITLE OF INVENTION: Helicobacter pylori live vaccine

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1557 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Helicobacter pylori

(vii) IMMEDIATE SOURCE:

(B) CLONE: alpB

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..1554

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATG ACA CAA TCT CAA AAA GTA AGA TTC TTA GCC CCT TTA AGC CTA GCG Met Thr Gln Ser Gln Lys Val Arg Phe Leu Ala Pro Leu Ser Leu Ala 1 5 10 15	48
TTA AGC TTG AGC TTC AAT CCA GTG GGC GCT GAA GAA GAT GGG GGC TTT Leu Ser Leu Ser Phe Asn Pro Val Gly Ala Glu Glu Asp Gly Gly Phe 20 25 30	96
ATG ACC TTT GGG TAT GAA TTA GGT CAG GTG GTC CAA CAA GTG AAA AAC Met Thr Phe Gly Tyr Glu Leu Gly Gln Val Val Gln Gln Val Lys Asn 35 40 45	144
CCG GGT AAA ATC AAA GCC GAA TTA GCC GGC TTG TTA AAC TCT ACC Pro Gly Lys Ile Lys Ala Glu Glu Leu Ala Gly Leu Leu Asn Ser Thr 50 55 60	192
ACA ACA AAC AAC ACC AAT ATC AAT ATT GCA GGC ACA GGA GGC AAT GTC	240

Thr	Thr	Asn	Asn	Thr	Asn	Ile	Asn	Ile	Ala	Gly	Thr	Gly	Gly	Asn	Val	
65				70					75					80		
GCC	GGG	ACT	TTG	GGC	<u>AAC</u>	CTT	TTT	ATG	AAC	CAA	TTA	GGC	AAT	TTG	ATT	288
Ala	Gly	Thr	Leu	Gly	Asn	Leu	Phe	Met	Asn	Gln	Leu	Gly	Asn	Leu	Ile	
															85	
															90	
															95	
GAT	TTG	TAT	CCC	ACT	TTG	AAC	ACT	AGT	AAT	ATC	ACA	CAA	TGT	GGC	ACT	336
Asp	Leu	Tyr	Pro	Thr	Leu	Asn	Thr	Ser	Asn	Ile	Thr	Gln	Cys	Gly	Thr	
															100	
															105	
															110	
ACT	AAT	AGT	GGT	AGT	AGT	AGT	GGT	GGT	GGT	GCG	GCC	ACA	GCC	GCT		384
Thr	Asn	Ser	Gly	Ser	Ser	Ser	Gly	Gly	Gly	Ala	Ala	Ala	Thr	Ala	Ala	
															115	
															120	
															125	
GCT	ACT	ACT	AGC	AAT	AAG	CCT	TGT	TTC	CAA	GGT	AAC	CTG	GAT	CTT	TAT	432
Ala	Thr	Thr	Ser	Asn	Lys	Pro	Cys	Phe	Gln	Gly	Asn	Leu	Asp	Leu	Tyr	
															130	
															135	
															140	
AGA	AAA	ATG	GTT	GAC	TCT	ATC	AAA	ACT	TTG	AGT	CAA	AAC	ATC	AGC	AAG	480
Arg	Lys	Met	Val	Asp	Ser	Ile	Lys	Thr	Leu	Ser	Gln	Asn	Ile	Ser	Lys	
															1	
															150	
															155	
AAT	ATC	TTT	CAA	GGC	AAC	AAC	ACC	ACG	AGC	CAA	AAT	CTC	TCC	TCC	AAC	528
Asn	Ile	Phe	Gln	Gly	Asn	Asn	Asn	Thr	Thr	Ser	Gln	Asn	Leu	Ser	Asn	
															165	
															170	
															175	
CAG	CTC	AGT	GAG	CTT	AAC	ACC	GCT	AGC	GTT	TAT	TTG	ACT	TAC	ATG	AAC	576
Gln	Leu	Ser	Glu	Leu	Asn	Thr	Ala	Ser	Val	Tyr	Leu	Thr	Tyr	Met	Asn	
															180	
															185	
															190	
TCG	TTC	TTA	AAC	GCC	AAT	AAC	CAA	GCG	GGT	GGG	ATT	TTT	CAA	AAC	AAC	624
Ser	Phe	Leu	Asn	Ala	Asn	Asn	Gln	Ala	Gly	Gly	Ile	Phe	Gln	Asn	Asn	
															195	
															200	
															205	
ACT	AAT	CAA	GCT	TAT	GGA	AAT	GGG	GTT	ACC	GCT	CAA	CAA	ATC	GCT	TAT	672
Thr	Asn	Gln	Ala	Tyr	Gly	Asn	Gly	Val	Thr	Ala	Gln	Gln	Ile	Ala	Tyr	
															210	
															215	
															220	
ATC	CTA	AAG	CAA	GCT	TCA	ATC	ACT	ATG	GGG	CCA	AGC	GGT	GAT	AGC	GGT	720
I	Leu	Lys	Gln	Ala	Ser	Ile	Thr	Met	Gly	Pro	Ser	Gly	Asp	Ser	Gly	
2..															230	
															235	
															240	
GCT	GCC	GCA	GCG	TTT	TTG	GAT	GCC	GCT	TTA	GCG	CAA	CAT	GTT	TTC	AAC	768
Ala	Ala	Ala	Ala	Phe	Leu	Asp	Ala	Ala	Leu	Ala	Gln	His	Val	Phe	Asn	
															245	
															250	
															255	
TCC	GCT	AAC	GCC	GGG	AAC	GAT	TTG	AGC	GCT	AAG	GAA	TTC	ACT	AGC	TTG	816
Ser	Ala	Asn	Ala	Gly	Asn	Asp	Leu	Ser	Ala	Lys	Glu	Phe	Thr	Ser	Leu	
															260	
															265	
															270	
GTG	CAA	AAT	ATC	GTC	AAT	AAT	TCT	CAA	AAC	GCT	TTA	ACG	CTA	GCC	AAC	864
Val	Gln	Asn	Ile	Val	Asn	Asn	Ser	Gln	Asn	Ala	Leu	Thr	Leu	Ala	Asn	
															275	
															280	
															285	
AAC	GCT	AAC	ATC	AGC	AAT	TCA	ACA	GGC	TAT	CAA	GTG	AGC	TAT	GGC	GGG	912
Asn	Ala	Asn	Ile	Ser	Asn	Ser	Thr	Gly	Tyr	Gln	Val	Ser	Tyr	Gly	Gly	
															290	
															295	
															300	
AAT	ATT	GAT	CAA	GCG	CGA	TCT	ACC	CAA	CTA	TTA	AAC	AAC	ACC	ACA	AAC	960
Asn	Ile	Asp	Gln	Ala	Arg	Ser	Thr	Gln	Leu	Leu	Asn	Asn	Thr	Thr	Asn	
															305	
															310	
															315	
															320	
ACT	TTG	GCT	AAA	GTT	AGC	GCT	TTG	AAT	AAC	GAG	CTT	AAA	GCT	AAC	CCA	1008

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 518 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Thr Gln Ser Gln Lys Val Arg Phe Leu Ala Pro Leu Ser Leu Ala
1 5 10 15

Leu Ser Leu Ser Phe Asn Pro Val Gly Ala Glu Glu Asp Cys Gly Phe
20 25 30

Met Thr Phe Gly Tyr Glu Leu Gly Gln Val Val Gln Gln Val Lys Asn
35 40 45

Pro Gly Lys Ile Lys Ala Glu Glu Leu Ala Gly Leu Leu Asn Ser Thr
50 55 60

Thr Thr Asn Asn Thr Asn Ile Asn Ile Ala Gly Thr Gly Gly Asn Val
65 70 75 80

Ala Gly Thr Leu Gly Asn Leu Phe Met Asn Gln Leu Gly Asn Leu Ile
85 90 95

Asp Leu Tyr Pro Thr Leu Asn Thr Ser Asn Ile Thr Gln Cys Gly Thr
100 105 110

Thr Asn Ser Gly Ser Ser Ser Gly Gly Gly Ala Ala Thr Ala Ala
115 120 125

Ala Thr Thr Ser Asn Lys Pro Cys Phe Gln Gly Asn Leu Asp Leu Tyr
130 135 140

Arg Lys Met Val Asp Ser Ile Lys Thr Leu Ser Gln Asn Ile Ser Lys
145 150 155 160

Asn Ile Phe Gln Gly Asn Asn Asn Thr Thr Ser Gln Asn Leu Ser Asn
165 170 175

Gln Leu Ser Glu Leu Asn Thr Ala Ser Val Tyr Leu Thr Tyr Met Asn
180 185 190

Ser Phe Leu Asn Ala Asn Asn Gln Ala Gly Gly Ile Phe Gln Asn Asn
195 200 205

Thr Asn Gln Ala Tyr Gly Asn Gly Val Thr Ala Gln Gln Ile Ala Tyr
210 215 220

Leu Lys Gln Ala Ser Ile Thr Met Gly Pro Ser Gly Asp Ser Gly
225 230 235 240

Ala Ala Ala Ala Phe Leu Asp Ala Ala Leu Ala Gln His Val Phe Asn
245 250 255

Ser Ala Asn Ala Gly Asn Asp Leu Ser Ala Lys Glu Phe Thr Ser Leu
260 265 270

Val Gln Asn Ile Val Asn Asn Ser Gln Asn Ala Leu Thr Leu Ala Asn
275 280 285

Asn Ala Asn Ile Ser Asn Ser Thr Gly Tyr Gln Val Ser Tyr Gly Gly
290 295 300

Asn Ile Asp Gln Ala Arg Ser Thr Gln Leu Leu Asn Asn Thr Asn
305 310 315 320

Thr Leu Ala Lys Val Ser Ala Leu Asn Asn Glu Leu Lys Ala Asn Pro
325 330 335

Trp Leu Gly Asn Phe Ala Ala Gly Asn Ser Ser Gln Val Asn Ala Phe
340 345 350

Asn Gly Phe Ile Thr Lys Ile Gly Tyr Lys Gln Phe Phe Gly Glu Asn
 355 360 365

Lys Asn Val Gly Leu Arg Tyr Tyr Gly Phe Phe Ser Tyr Asn Gly Ala
 370 375 380

Gly Val Gly Asn Gly Pro Thr Tyr Asn Gln Val Asn Leu Leu Thr Tyr
 385 390 395 400

Gly Val Gly Thr Asp Val Leu Tyr Asn Val Phe Ser Arg Ser Phe Gly
 405 410 415

Ser Arg Ser Leu Asn Ala Gly Phe Phe Gly Gly Ile Gln Leu Ala Gly
 420 425 430

Asp Thr Tyr Ile Ser Thr Leu Arg Asn Ser Ser Gln Leu Ala Ser Arg
 435 440 445

Pro Thr Ala Thr Lys Phe Gln Phe Leu Phe Asp Val Gly Leu Arg Met
 450 455 460

A' Phe Gly Ile Leu Lys Lys Asp Leu Lys Ser His Asn Gln His Ser
 465 470 475 480

Ile Glu Ile Gly Val Gln Ile Pro Thr Ile Tyr Asn Thr Tyr Tyr Lys
 485 490 495

Ala Gly Gly Ala Glu Val Lys Tyr Phe Arg Pro Tyr Ser Val Tyr Trp
 500 505 510

Val Tyr Gly Tyr Ala Phe
 515

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1557 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Helicobacter pylori

(vii) IMMEDIATE SOURCE:

- (B) CLONE: alpA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1554

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATG ATA AAA AAG AAT AGA ACG CTG TTT CTT AGT CTA GCC CTT TGC GCT
 Met Ile Lys Lys Asn Arg Thr Leu Phe Leu Ser Leu Ala Leu Cys Ala
 520 525 530

AGC ATA AGT TAT GCC GAA GAT GAT GGA GGG TTT TTC ACC GTC GGT TAT
 Ser Ile Ser Tyr Ala Glu Asp Asp Gly Gly Phe Phe Thr Val Gly Tyr
 535 540 545 550

48

96

44

CAG CTC GGG CAA GTC ATG CAA GAT GTC CAA ACG CCA GGC GGC GCT AAA Gln Leu Gly Gln Val Met Gln Asp Val Gln Asn Pro Gly Gly Ala Lys 555 560 565	
AGC GAC GAA CTC GCC AGA GAG CTT AAC GCT GAT GTA ACG AAC AAC ATT Ser Asp Glu Leu Ala Arg Glu Leu Asn Ala Asp Val Thr Asn Asn Ile 570 575 580	192
TTA AAC AAC ACC GGA GGC AAC ATC GCA GGG GCG TTG AGT AAC GCT Leu Asn Asn Thr Gly Gly Asn Ile Ala Gly Ala Leu Ser Asn Ala 585 590 595	240
TTC TCC CAA TAC CTT TAT TCG CTT TTA GGG GCT TAC CCC ACA AAA CTC Phe Ser Gln Tyr Leu Tyr Ser Leu Leu Gly Ala Tyr Pro Thr Lys Leu 600 605 610	288
AAT GGT AGC GAT GTG TCT GCG AAC GCT CTT TTA AGT GGT GCG GTA GGC Asn Gly Ser Asp Val Ser Ala Asn Ala Leu Leu Ser Gly Ala Val Gly 615 620 625 630	336
TCT GGG ACT TGT GCG GCT GCA GGG ACG GCT GGT GGC ACT TCT CTT AAC S Gly Thr Cys Ala Ala Ala Gly Thr Ala Gly Gly Thr Ser Leu Asn 635 640 645	384
ACT CAA AGC ACT TGC ACC GTT GCG GGC TAT TAC TGG CTC CCT AGC TTG Thr Gln Ser Thr Cys Thr Val Ala Gly Tyr Tyr Trp Leu Pro Ser Leu 650 655 660	432
ACT GAC AGG ATT TTA AGC ACG ATC GGC AGC CAG ACT AAC TAC GGC ACG Thr Asp Arg Ile Leu Ser Thr Ile Gly Ser Gln Thr Asn Tyr Gly Thr 665 670 675	480
AAC ACC AAT TTC CCC AAC ATG CAA CAA CAG CTC ACC TAC TTG AAT GCG Asn Thr Asn Phe Pro Asn Met Gln Gln Gln Leu Thr Tyr Leu Asn Ala 680 685 690	528
GGG AAT GTG TTT TTT AAT GCG ATG AAT AAG GCT TTA GAG AAT AAG AAT Gly Asn Val Phe Phe Asn Ala Met Asn Lys Ala Leu Glu Asn Lys Asn 695 700 705 710	576
ACT AGT AGT GCT AGT GGA ACT AGT GGT GCG ACT GGT TCA GAT GGT Gly Thr Ser Ser Ala Ser Gly Thr Ser Gly Ala Thr Gly Ser Asp Gly 715 720 725	624
CAA ACT TAC TCC ACA CAA GCT ATC CAA TAC CTT CAA GGC CAA CAA AAT Gln Thr Tyr Ser Thr Gln Ala Ile Gln Tyr Leu Gln Gly Gln Gln Asn 730 735 740	672
ATC TTA AAT AAC GCA GCG AAC TTG CTC AAG CAA GAT GAA TTG CTC TTA Ile Leu Asn Asn Ala Ala Asn Leu Leu Lys Gln Asp Glu Leu Leu Leu 745 750 755	720
GAA GCT TTC AAC TCT GCC GTA GCC GCC AAC ATT GGG AAT AAG GAA TTC Glu Ala Phe Asn Ser Ala Val Ala Ala Asn Ile Gly Asn Lys Glu Phe 760 765 770	768
AAT TCA GCC GCT TTT ACA GGT TTG GTG CAA GGC ATT ATT GAT CAA TCT Asn Ser Ala Ala Phe Thr Gly Leu Val Gln Gly Ile Ile Asp Gln Ser 775 780 785 790	816
CAA GCG GTT TAT AAC GAG CTC ACT AAA AAC ACC ATT AGC GGG AGT GCG Gln Ala Val Tyr Asn Glu Leu Thr Lys Asn Thr Ile Ser Gly Ser Ala 795 800 805	864

912

GTT ATT AGC GCT GGG ATA AAC TCC AAC CAA GCT AAC GCT GTG CAA GGG Val Ile Ser Ala Gly Ile Asn Ser Asn Gln Ala Asn Ala Val Gin Gly	810	815	820	
CGC GCT AGT CAG CTC CCT AAC GCT CTT TAT AAC GCG CAA GTA ACT TTG Arg Ala Ser Gln Leu Pro Asn Ala Leu Tyr Asn Ala Gln Val Thr Leu	825	830	835	960
GAT AAA ATC AAT GCG CTC AAT AAT CAA GTG AGA AGC ATG CCT TAC TTG Asp Lys Ile Asn Ala Leu Asn Asn Gln Val Arg Ser Met Pro Tyr Leu	840	845	850	1008
CCC CAA TTC AGA GCC GGG AAC AGC CGT TCA ACG AAT ATT TTA AAC GGG Pro Gln Phe Arg Ala Gly Asn Ser Arg Ser Thr Asn Ile Leu Asn Gly	855	860	865	1056
TTT TAC ACC AAA ATA GGC TAT AAG CAA TTC TTC GGG AAG AAA AGG AAT Phe Tyr Thr Lys Ile Gly Tyr Lys Gln Phe Phe Gly Lys Lys Arg Asn	875	880	885	1104
ATC GGT TTG CGC TAT TAT GGT TTC TTT TCT TAT AAC GGA GCG AGC GTG Gly Leu Arg Tyr Tyr Gly Phe Ser Tyr Asn Gly Ala Ser Val	890	895	900	1152
GGC TTT AGA TCC ACT CAA AAT AAT GTC GGG TTA TAC ACT TAT GGG GTG Gly Phe Arg Ser Thr Gln Asn Asn Val Gly Leu Tyr Thr Gly Val	905	910	915	1200
GGG ACT GAT GTG TTG TAT AAC ATC TTT AGC CGC TCC TAT CAA AAC CGC Gly Thr Asp Val Leu Tyr Asn Ile Phe Ser Arg Ser Tyr Gln Asn Arg	920	925	930	1248
TCT GTG GAT ATG GGC TTT TTT AGC GGT ATC CAA TTA GCC GGT GAG ACC Ser Val Asp Met Gly Phe Phe Ser Gly Ile Gln Leu Ala Gly Glu Thr	935	940	945	1296
TTC CAA TCC ACG CTC AGA GAT GAC CCC AAT GTG AAA TTG CAT GGG AAA Phe Gln Ser Thr Leu Arg Asp Asp Pro Asn Val Lys Leu His Gly Lys	955	960	965	1344
AAT AAC ACG CAC TTC CAG TTC CTC TTT GAC TTC GGT ATG AGG ATG Ile Asn Asn Thr His Phe Gln Phe Leu Phe Asp Phe Gly Met Arg Met	970	975	980	1392
AAC TTC GGT AAG TTG GAC GGG AAA TCC AAC CGC CAC AAC CAG CAC ACG Asn Phe Gly Lys Leu Asp Gly Lys Ser Asn Arg His Asn Gln His Thr	985	990	995	1440
GTG GAA TTT GGC GTA GTG GTG CCT ACG ATT TAT AAC ACT TAT TAC AAA Val Glu Phe Gly Val Val Pro Thr Ile Tyr Asn Thr Tyr Tyr Lys	1000	1005	1010	1488
TCA GCA GGG ACT ACC GTG AAG TAT TTC CGT CCT TAT AGC GTT TAT TGG Ser Ala Gly Thr Thr Val Lys Tyr Phe Arg Pro Tyr Ser Val Tyr Trp	1015	1020	1025	1536
TCT TAT GGG TAT TCA TTC TAA Ser Tyr Gly Tyr Ser Phe	1035			1557

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 518 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Ile Lys Lys Asn Arg Thr Leu Phe Leu Ser Leu Ala Leu Cys Ala
1 5 10 15

Ser Ile Ser Tyr Ala Glu Asp Asp Gly Gly Phe Phe Thr Val Gly Tyr
20 25 30

Gln Leu Gly Gln Val Met Gln Asp Val Gln Asn Pro Gly Gly Ala Lys
35 40 45

Ser Asp Glu Leu Ala Arg Glu Leu Asn Ala Asp Val Thr Asn Asn Ile
50 55 60

Leu Asn Asn Asn Thr Gly Gly Asn Ile Ala Gly Ala Leu Ser Asn Ala
65 70 75 80

Phe Ser Gln Tyr Leu Tyr Ser Leu Leu Gly Ala Tyr Pro Thr Lys Leu
85 90 95

Asn Gly Ser Asp Val Ser Ala Asn Ala Leu Leu Ser Gly Ala Val Gly
100 105 110

Ser Gly Thr Cys Ala Ala Ala Gly Thr Ala Gly Gly Thr Ser Leu Asn
115 120 125

Thr Gln Ser Thr Cys Thr Val Ala Gly Tyr Tyr Trp Leu Pro Ser Leu
130 135 140

Thr Asp Arg Ile Leu Ser Thr Ile Gly Ser Gln Thr Asn Tyr Gly Thr
145 150 155 160

Asn Thr Asn Phe Pro Asn Met Gln Gln Gln Leu Thr Tyr Leu Asn Ala
165 170 175

Asn Val Phe Phe Asn Ala Met Asn Lys Ala Leu Glu Asn Lys Asn
180 185 190

Gly Thr Ser Ser Ala Ser Gly Thr Ser Gly Ala Thr Gly Ser Asp Gly
195 200 205

Gln Thr Tyr Ser Thr Gln Ala Ile Gln Tyr Leu Gln Gly Gln Gln Asn
210 215 220

Ile Leu Asn Asn Ala Ala Asn Leu Leu Lys Gln Asp Glu Leu Leu Leu
225 230 235 240

Glu Ala Phe Asn Ser Ala Val Ala Ala Asn Ile Gly Asn Lys Glu Phe
245 250 255

Asn Ser Ala Ala Phe Thr Gly Leu Val Gln Gly Ile Ile Asp Gln Ser
260 265 270

Gln Ala Val Tyr Asn Glu Leu Thr Lys Asn Thr Ile Ser Gly Ser Ala
275 280 285

Val Ile Ser Ala Gly Ile Asn Ser Asn Gln Ala Asn Ala Val Gln Gly
290 295 300

Arg Ala Ser Gln Leu Pro Asn Ala Leu Tyr Asn Ala Gln Val Thr Leu
305 310 315 320

Asp Lys Ile Asn Ala Leu Asn Asn Gln Val Arg Ser Met Pro Tyr Leu
325 330 335

Pro Gln Phe Arg Ala Gly Asn Ser Arg Ser Thr Asn Ile Leu Asn Gly
340 345 350

Phe Tyr Thr Lys Ile Gly Tyr Lys Gln Phe Phe Gly Lys Lys Arg Asn
355 360 365

Ile Gly Leu Arg Tyr Tyr Gly Phe Phe Ser Tyr Asn Gly Ala Ser Val
370 375 380

Gly Phe Arg Ser Thr Gln Asn Asn Val Gly Leu Tyr Thr Tyr Gly Val
385 390 395 400

Gly Thr Asp Val Leu Tyr Asn Ile Phe Ser Arg Ser Tyr Gln Asn Arg
405 410 415

S Val Asp Met Gly Phe Phe Ser Gly Ile Gln Leu Ala Gly Glu Thr
420 425 430

Phe Gln Ser Thr Leu Arg Asp Asp Pro Asn Val Lys Leu His Gly Lys
435 440 445

Ile Asn Asn Thr His Phe Gln Phe Leu Phe Asp Phe Gly Met Arg Met
450 455 460

Asn Phe Gly Lys Leu Asp Gly Lys Ser Asn Arg His Asn Gln His Thr
465 470 475 480

Val Glu Phe Gly Val Val Val Pro Thr Ile Tyr Asn Thr Tyr Tyr Lys
485 490 495

Ser Ala Gly Thr Thr Val Lys Tyr Phe Arg Pro Tyr Ser Val Tyr Trp
500 505 510

Ser Tyr Gly Tyr Ser Phe
515

Patent Claims

1. A recombinant attenuated microbial pathogen, which comprises at least one heterologous nucleic acid molecule encoding a *Helicobacter* antigen, wherein said pathogen is capable to express said nucleic acid molecule or capable to cause the expression of said nucleic acid molecule in a target cell.
- 10 2. The pathogen according to claim 1, which is an enterobacterial cell, especially a *Salmonella* cell.
- 15 3. The pathogen according to claim 1 or 2, which is a *Salmonella* aro mutant cell.
4. The pathogen according to any of claims 1-3, wherein the *Helicobacter* antigen is urease, a urease subunit, an immunologically reactive fragment thereof, or a peptide mimotope thereof.
- 20 5. The pathogen according to any one of claims 1-3, wherein the *Helicobacter* antigen is a secretory polypeptide from *Helicobacter*, an immunologically reactive fragment thereof, or a peptide mimotope thereof.
- 25 6. The pathogen according to any one of claims 1-3 and 5, wherein the *Helicobacter* antigen is selected from the group consisting of the antigens AlpA, AlpB, immunologically reactive fragments thereof, or a peptide mimotope thereof.
- 30 7. The pathogen according to any one of claims 1-6, wherein said nucleic acid molecule encoding a *Helicobacter* antigen is capable to be expressed phase variably.
- 35 8. The pathogen according to claim 7,

wherein said nucleic acid molecule encoding the Helicobacter antigen is under control of an expression signal which is substantially inactive in the pathogen and which is capable to be activated by a nucleic acid reorganization caused by a nucleic acid reorganization mechanism in the pathogen.

- 5 9. The pathogen according to claim 8,
wherein the expression signal is a bacteriophage promoter,
10 and the activation is caused by a DNA reorganization resulting in the production of a corresponding bacteriophage RNA polymerase in the pathogen.
- 15 10. The pathogen according to any one of claims 1-9, further comprising at least one second nucleic acid molecule encoding an immunomodulatory polypeptide, wherein said pathogen is capable to express said second nucleic acid molecule.
- 20 11. Pharmaceutical composition comprising as an active agent a recombinant attenuated pathogen according to any one of claims 1-10, optionally together with pharmaceutically acceptable diluents, carriers and adjuvants.
- 25 12. Composition according to claim 11, which is a living vaccine, which is suitable for administration to a mucosal surface or via the parenteral route.
- 30 13. A method for the preparation of a living vaccine comprising formulating an attenuated pathogen according to any one of claims 1-10 in a pharmaceutically effective amount with pharmaceutically acceptable diluents, carriers and/or adjuvants.
- 35 14. A method for preparing a recombinant attenuated pathogen according to any one of claims 1-10, comprising the steps:

- a) inserting a nucleic acid molecule encoding a Helicobacter antigen into an attenuated pathogen, wherein a recombinant attenuated pathogen is obtained, which is capable of expressing said nucleic acid molecule or is capable to cause expression of said nucleic acid molecule in a target cell, and
- b) cultivating said recombinant attenuated pathogen under suitable conditions.

10 15. The method according to claim 15,
wherein said nucleic acid molecule encoding a Helicobacter antigen is located on an extrachromosomal plasmid.

15 16. A method for identifying Helicobacter antigens, which raise a protective immune response in a mammalian host, comprising the steps of:

- a) providing an expression gene bank of Helicobacter in an attenuated pathogen and
- b) screening the clones of the gene bank for their ability to confer protective immunity against a Helicobacter infection in a mammalian host.

25

30

Abstract

The present invention relates to novel recombinant live vaccines, which provide protective immunity against an infection by Helicobacter pylori and a method of screening H. pylori antigens for optimized vaccines.

10

/users/ff/chem/15258PEP.anm 11.10.1996

1/2
11,617,200 6

FIG. 1

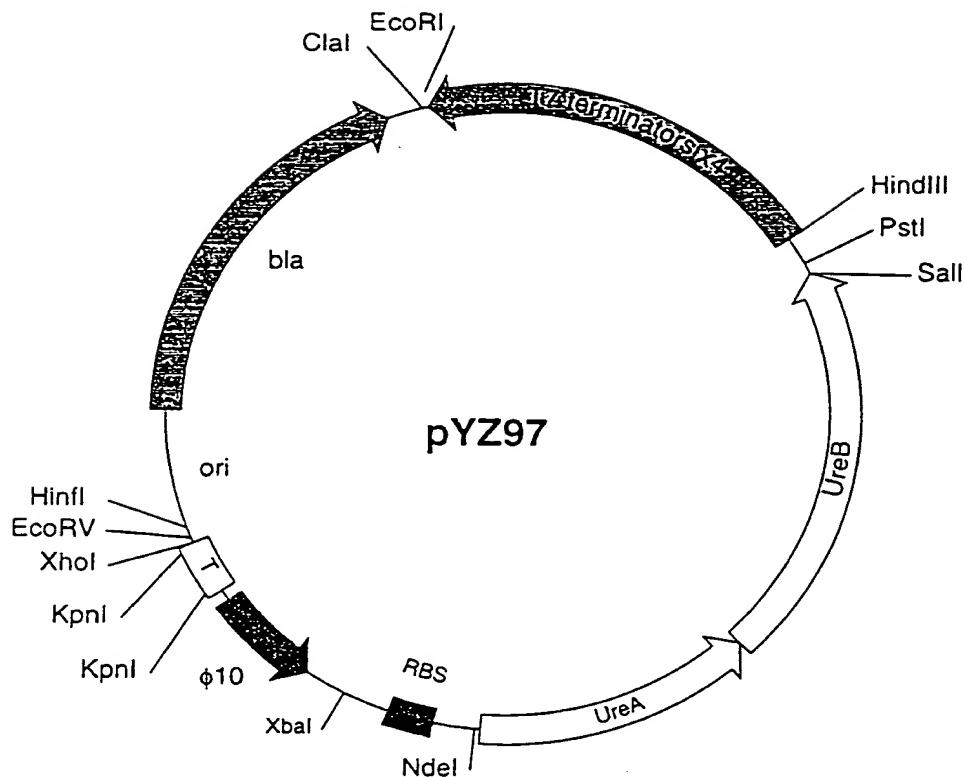
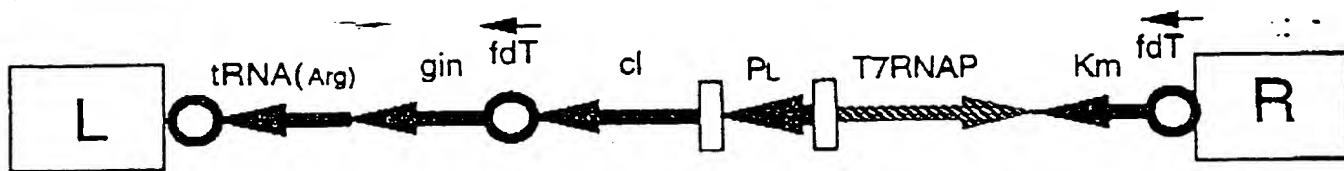
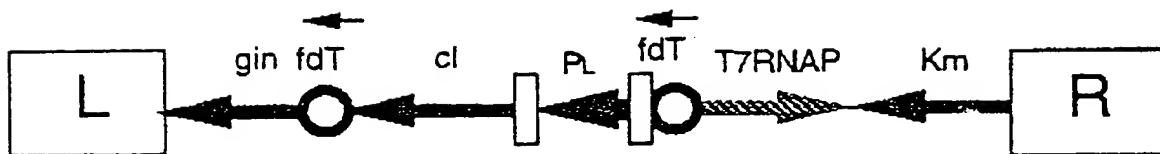


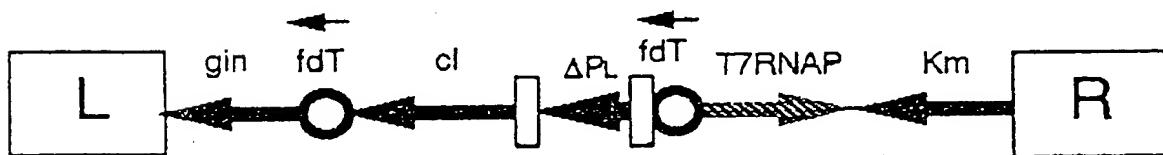
FIG. 2



pYZ88 (high expression)



pYZ84 (medium expression)



pYZ114 (low expression)